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Bacterial Flora in Spontaneously Occurring Aural Cholesteatomas in Mongolian Gerbils

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Bacteria were isolated from 29 Mongolian gerbils, *Meriones unguiculatus*, with spontaneous aural cholesteatomas. We identified 148 cultures, 44 from the middle ear surface of cholesteatomas and 104 from cholesteatoma contents. We could only identify 63 cultures to the genus level, although we identified 85 cultures as belonging to 21 different species. We found on the surfaces of cholesteatomas representatives of 9 genera, from which 8 species could be identified, and representatives of 19 genera within the cholesteatoma sac, from which 21 species could be identified. The most common bacterial genera isolated were *Staphylococcus*, *Pseudomonas*, and *Corynebacterium* among the aerobic genera, and *Bacteroides* and *Peptococcus* among the anaerobic genera. The bacterial flora of gerbilline cholesteatomas was found to be diverse, resembling the flora found to be associated with human cholesteatomas. The flora also resembled the organisms found transitorily within the normal middle ear cavity of gerbils, except for a higher incidence of pseudomonads.

Aural cholesteatomas develop spontaneously in aging Mongolian gerbils, *Meriones unguiculatus* (4). These cholesteatomas enlarge from the tympanic membrane and ear canal into the middle ear and bulla. Contact between the cholesteatoma and subjacent bone results in bone erosion. Otitis media has been seen in association with some of the more advanced cholesteatomas, and bacteria have been seen invariably within the keratin of most cholesteatomas (3). Bacteria are also reported to be associated with human cholesteatomas (1, 2, 8, 12, 13).

Gerbils without cholesteatomas have been shown to be a suitable model for otitis media, since their middle ears contain few bacteria (22), and otitis media may be readily induced by inoculation with *Streptococcus pneumoniae* and *Haemophilus influenzae* (6, 7, 10; R. S. Fulghum, R. P. Hoogmoed, J. E. Brinn, and A. M. Smith, Int. J. Pediatr. Otorhinolaryngol., in press). The microbiological flora of spontaneous gerbilline cholesteatomas is unknown, as is the microbiology of spontaneous otitis media in some gerbils with cholesteatomas.

The purpose of this study was to identify the species of bacteria present on the middle ear surface of cholesteatomas and within the cholesteatoma sac.

MATERIALS AND METHODS

Bacteria were isolated from 29 Mongolian gerbils, *Meriones unguiculatus*, which had spontaneous aural cholesteatomas. There animals were either retired breeding stock obtained from Tumblebrook Farm or first-generation offspring of breeders from the same source. The animals were of both sexes and were between 1 and 3 years of age.

Each animal was killed by a lethal dose of pentobarbital. The bullae were entered from the ventral surface by the removal of fur and the swabbing of the overlying skin with betadine, after which the skin was incised and a window was resected from the bulla. A small sterile loop was used to obtain a specimen from the surface of the cholesteatoma in the middle ear. Next, the matrix (thin epithelial covering) of the cholesteatoma was opened and a sample was taken from the contents of the cholesteatoma with a sterile Pasteur pipette or with a sterile loop.

Samples were then placed immediately in an anaerobic tube containing a small amount of prereduced, anaerobically sterilized brain heart infusion-supplemented broth medium (9) and were transported quickly (within 10 min) to the bacteriology laboratory. Loopfuls of the specimen in prereduced, anaerobically sterilized brain heart infusion-supplemented broth medium were streaked using the methods of Hungate (11) as modified by Moore (19) in prereduced, anaerobically sterilized brain heart infusion-supplemented roll tubes and in prereduced, anaerobically sterilized brain heart infusion-supplemented roll tubes containing the following metabolic products and intermediates: formic, fumaric, lactic, and pyruvic acids at a 0.03M concentration for anaerobic incubation. Commercially prepared sheep blood agar and chocolate agar plates were streaked for aerobic incubation. After 48 h of incubation all tubes were checked, and colonies appearing were isolated in pure culture. Anaerobic tubes were reincubated, and any further growth was isolated after 5 days of incuabtion. Growth on aerobic plates was isolated in pure cultures after 2 days of incubation. Where numerous colonies appeared from a specimen, only representative members of like colonies were isolated in pure cultures for identification.

Anaerobic bacteria were identified by using the methods and criteria of the Virginia Polytechnic Institute Anaerobe Laboratory (9), and aerobic isolates were identified by using API 20E or API Staph-Ident (Analytab Products, Plainview, N.Y.) commercial identification systems or customary methods (15). Data obtained for the gram-negative species were also compared with the data found in *Bergey's Manual of Systematic Bacteriology* (14).

RESULTS

We identified 148 cultures, 44 from the middle ear surface of cholesteatomas and 104 from cholesteatoma contents. We

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TABLE 1. Bacteria isolated from cholesteatomas of gerbils

Metabolism	Gram reaction	Morphology	Bacteria	No. of cultures from: Cholesteatoma contents[a]	No. of cultures from: Middle ear surfaces[b]
Aerobic or facultatively anaerobic	+	Coccus	*Staphylococcus aureus*	15	8
			Staphylococcus epidermidis	5	
			Staphylococcus haemolyticus	1	
			Staphylococcus saprophyticus	1	1
			Staphylococcus simulans	2	2
			Staphylococcus sciuri	1	
			Staphylococcus	10	7
			Streptococcus spp. group D	3	
			Streptococcus spp.	2	2
Aerobic	+	Rod	*Corynebacterium aquaticum*	1	
			Corynebacterium pseudodiphtheriticum	1	
			Corynebacterium spp.	8	2
			Lactobacillus spp.	2	
			Lactobacillus plantarum	1	
Aerobic	−	Coccus	*Moraxella* spp.	1	2
Aerobic	−	Rod	*Acinetobacter calcoaceticus*	5	2
			Escherichia coli	7	3
			Pasteurella or *Actinobacillus* spp.	2	
			Pseudomonas paucimobilis	1	
			Pseudomonas aeruginosa	5	4
			Pseudomonas spp.	6	4
			Fluorescent *Pseudomonas* group	4	1
			Yersinia pseudotuberculosis	1	
			Yersinia spp.	2	
			Citrobacter freundii	4	
			Chromobacterium spp.	1	
Anaerobic	+	Coccus	*Peptococcus magnus*	2	4
			Peptococcus indolicus	1	
Anaerobic	+	Rod	*Propionibacterium avidum*	1	
			Clostridium subterminale	1	
Anaerobic	−	Rod	*Bacteroides capillosus*	2	2
			Bacteroides spp.	1	
			Fusobacterium gonidiaformans	1	
Other			*Cedecea* spp.	2	
			CDC group VE-1	1	

[a] From 29 gerbil cholesteatoma sac contents.
[b] From 24 gerbil middle ear cholesteatoma surfaces.

TABLE 2. Numerical distribution of specimens by numbers of organisms isolated per specimen

Nos. of genera and species/specimen	No. of specimens containing this no. of genera and species derived from: Contents of cholesteatoma sac[a]	No. of specimens containing this no. of genera and species derived from: Middle ear surface of cholesteatoma sac[b]
0 (Sterile)	2	10
1	2	1
2	3	4
3	5	4
4	8	4
5	6	0
6	2	1
7	1	0

[a] Total of 29 specimens.
[b] Total of 24 specimens.

found 9 genera from which 8 species could be identified on the surfaces of cholesteatomas and 19 genera from which 21 species could be identified within the cholesteatoma sac. A number of isolates could not be identified beyond the genus level. Although 85 cultures were identified as belonging to 21 different species, 63 cultures could be identified only to the genus level. The most common bacterial genera isolated were *Staphylococcus*, *Pseudomonas*, and *Corynebacterium* among the aerobic genera, and *Bacteroides* and *Peptococcus* among the anaerobic genera. Bacterial genera and species were found to be randomly distributed among the specimens, and no characteristic associations of species were found to characterize either the bacteria of the sac contents or the sac surfaces. The genera and species of bacteria isolated from the 29 gerbils studied are listed in Table 1.

From within the cholesteatoma sac, an average of 3.6 genera and species were isolated although an average of only 2.9 genera and species were found within the middle ear

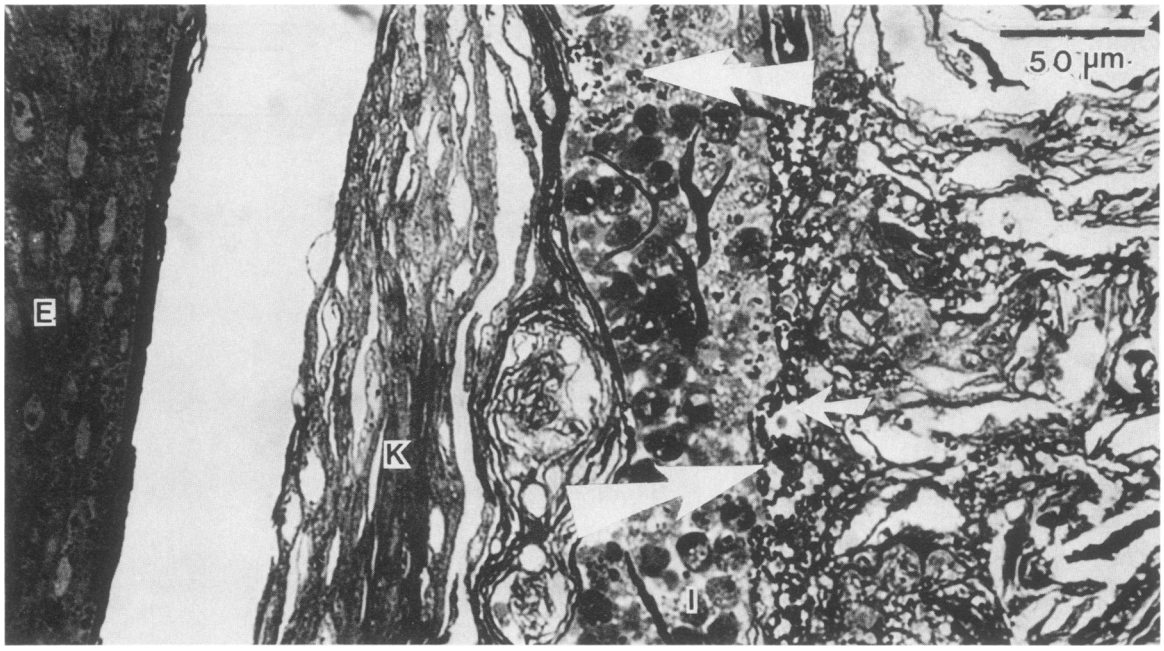

FIG. 1. Photomicrograph of a cholesteatoma with its epithelial sac (E) and keratin debris (K). Inflammatory cells (I) are seen in the lower center; bacteria (arrows) are seen in the top center and center.

cavity on the surface of the cholesteatoma. Only 2 of the 29 (7%) cholesteatoma sacs sampled were sterile, although 10 of the 24 (42%) sac surfaces of the middle ear were sterile. Table 2 presents the distribution of specimens by the numbers of genera and species per specimen. Figure 1 is a photomicroscopic picture of cholesteatoma tissue, showing bacteria within the sac material.

DISCUSSION

The bacterial flora of gerbilline cholesteatomas is similar to that found in humans (1, 2, 8, 13). Many of the species are the same as those found in the gerbilline nasopharyngeal flora and the transitory flora of the normal gerbilline middle ear cavity (22).

Although pathogenic bacteria are found within the flora of gerbilline cholesteatomas, they are not found in all cases. Therefore, no one organism can be associated with this condition. There is no consistent pattern of association among the members of the polymicrobial infection flora found on and in gerbilline cholesteatomas; therefore, no mixture of species or genera can be associated with aural cholesteatomas in gerbils.

Bacteria, bacterial products, and bacterial enzymes have been suggested as significant factors in bone resorption in human cholesteatomas (12). Other factors have been reported to be responsible for bone resorption in other body locations (5). Moriyama et al. (20) suggested that endotoxin lipopolysaccharide-activated macrophages in otitis media release prostaglandin E_2, which has been shown to stimulate bone resorption (21). Whether bacteria or their by-products are causally related to the development of cholesteatomas in gerbils is not known. We have seen gerbils in this study whose cholesteatomas have eroded the bone of the posterior portion of the middle ear bullae and were found under the skin and connective tissue. Others have been observed to invade the cochlea and the cranial cavity of gerbils (17). However, mechanical factors due to the pressure of the invading cholesteatoma may induce bone resorption in much the same fashion that pressure on the teeth by orthodontic procedures causes resorption and disposition of bone in the jaws. Macri and Chole (16) found that the presence of impermeable barriers between cholesteatomas and bone did not inhibit bone resorption. It is possible that a combination of mechanical, biological, exogenous, and endogenous factors produces the effect. Bone resorption was seen in most of the animals in our study. The degree of bone resorption appeared to be correlated with the size of the cholesteatoma, as previously observed (4). The degree of bone resorption was not assessed, since the cholesteatoma sac was removed for culture and the underlying bone and cochlea were prepared for a subsequent scanning electron microscope study of hair cells.

Schmiedt (personal communication) has suggested that blockage of the external auditory canal in younger gerbils may be caused by mechanical irritation due to bits of bedding and hair falling into the external auditory canal and lodging against the tympanic membrane. We also have observed large amounts of cerumen and keratin produced, surrounding debris in the external auditory canal of such animals. Presumably, this continues until blockage occurs and could be a step in the development of a cholesteatoma. It is known that keratin debris forms on the pars flaccida first and that blockage of the canal is a late event. Ligation of the external auditory canal is known to induce cholesteatomas (17, 18).

In summary, we find the bacterial flora of gerbilline cholesteatomas to be diverse, with no consistent membership of species found in all cholesteatomas. It resembles the flora associated with human cholesteatomas. The flora is not unlike the list of transitory organisms found in the normal gerbil middle ear cavity (22) except for the higher incidence of pseudomonads found in many of the animals with cholesteatomas.

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